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14. ABSTRACT

To determine antitumor immunity in MMT mice that are deficient for telomerase activity, we immunized MMT, G0 and G1 to G4 mTERC–/–MMT mice with DC/tumor fusion vaccine (FC/MMT). Vaccination of MMT, G0 and G1 to G4 mTERC–/–MMT mice induced CTL that lysed MUC1-positive tumor cells, suggesting that the cellular immunity is not affected by telomerase inactivity, at least in the G1 and G2 mTERC–/–MMT mice. The induction of CTL in these mice translated into delayed appearance of mammary carcinomas. The latency time for the mammary tumors in MMT, G0 and G1 to G4 mTERC–/–MMT mice immunized with FC/MUC1 were 112.4±10.4, 113.6±8.4, 136.3±10, 144.3±12.7, 158.7±15.7 and 247.3 days, respectively. The difference of latency time between immunized and non-immized mice was statistically significant in all groups except the G4 group. In addition, T cell proliferation and cell division in G1 and G3 mTERC–/–MMT mice were comparable with those from MMT and G0 mice as demonstrated by standard isotope incorporation and CFSE labeling. Taken together, these results indicate that telomerase inactivity enhances the antitumor immunity, at least in the first and second generations of mTERC–/–MMT mice.

15. SUBJECT TERMS

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INTRODUCTION:

One of the characteristics of malignant cells is their unlimited capacity of proliferation. Normal cells divide for a limited number of times and then enter cellular senescence, whereas tumor cells can proliferate indefinitely and became immortal (1). Studies show that cellular senescence exists in all mammal cells but escaped in tumor cells through various mechanisms. One of the mechanisms is the expression of telomerase in tumor cells. Indeed, telomerase is expressed in approximately 90% of human malignant tumors and plays a significant role in the development, progression and metastasis of malignant tumors including breast cancer. Therefore, telomerase represents a potential target for cancer therapy. Telomerase can be inhibited by a variety of agents such as dominant-negative hTert (2, 3), antisense oligonucleotides (4, 5), RNA interference (6) or some small-molecule inhibitors interacting with the G-quadruplexes of telomerase (7, 8). Most studies, however, have been limited to the use of cultured cells due to lack of an applicable animal model. An *in vivo* model that closely mimics human cancer is desirable for studying the antitelomerase effect on tumor cells as well as on normal tissues. In our lab, we generated mice in the C57BL/6 strain carrying the polyoma virus middle T oncogene (PyMT) but deficient for mTerc by crossing mTerc$^{-/-}$ mice with MMT mice that develop spontaneous mammary carcinomas (9, 10). Our initial results show that tumorigenesis of mammary carcinoma is severely compromised by telomerase deficiency. Tumors still developed in G3 mTerc$^{-/-}$MMT mice, albeit with much reduced tumor burden. The reduced tumor burden may create a favorable condition for other therapies. In the proposed study, immunotherapy is used since it has minimal toxicity to the host and works most effectively in the setting of minimal tumor burden. Successive generations of MMT mice with deficiency of telomerase activity were generated and used to test the efficacy of immunotherapy in the prevention and treatment of mammary carcinomas in the background of telomerase deficiency. In addition, our model system allows us to assess whether inhibition of telomerase activity has a detrimental effect on antitumor immunity. Such knowledge is not available in the literature.

BODY:

The proposed study is to explore the combined approaches of telomerase inhibition and immunotherapy in the prevention and treatment of mammary carcinoma. In our previous studies, we have generated MMT mice that develop spontaneous mammary carcinomas. More important, the development of mammary carcinomas is correlated with the telomerase activity. These results indicate that telomerase activity plays a role in the formation or progression of mammary tumors, suggesting that telomerase is a potential target in the prevention or treatment of mammary carcinomas. The specific aims of the proposed study remains the same: (i) to determine the effect of telomerase deficiency on both tumorigenesis and the functioning of highly proliferative normal organs; (ii) to determine the synergistic effect in the management of mammary carcinomas by combined immunotherapy and depletion of telomerase activity. In 2007, we generated MMT mice with deficient telomerase activity (mTerc$^{-/-}$MMT mice) for 3 generations. The mice were immunized with fusions of dendritic cells (DC) and MUC1-positive mammary tumor cells (FC/MMT). The immune responses to the vaccine in each generation of mTerc$^{-/-}$MMT mice including latency time, T cell proliferation and induction of CTL were determined. In 2008, more G1 to G3 mTerc$^{-/-}$MMT mice were

generated and used in the experiment. In addition, G4 mTerc$^{-/-}$MMT mice were also generated. Overall, the tasks outlined in Specific Aim 1 have been fulfilled as planned. The majority of tasks outlined in Specific Aim 2 have been carried out although some mice are still under observation, especially the late generations of mTerc$^{-/-}$MMT mice (G3 and G4). No-cost extension for one year is needed to finish the project.

Task 1. **To determine the effect of telomerase deficiency on both tumorigenesis and function of highly proliferative normal organs**

Effect of telomerase deficiency on tumorigenesis. To assess the role of telomerase in the tumorigenesis of mammary carcinomas, successive generational intercrosses of mTerc$^{-/-}$MMT mice produced cohorts with progressively shorter telomeres that were audited for mammary tumor formation. Littermates with different genotypes including MMT and G0 mTerc$^{+/-}$MMT mice were used as controls. In the previous report, we observed a trend for the tumor development in mTerc$^{-/-}$MMT mice: successive generations of mTerc$^{-/-}$MMT mice were associated with increased latency time for tumor formation and a decrease in numbers and volume of mammary tumors. The impairment of tumorigenesis in mTerc$^{-/-}$MMT mice was also indicated by the histological examination. Whole Mount examination reveals multiple invasive mammary tumors in MMT and G0 mTerc$^{+/-}$MMT mice (Fig 1A). By contrast, mammary tumor formation was inversely correlated with the number of generations of mTerc$^{-/-}$MMT mice. The formation of primary tumors as well as secondary tumor masses was significantly decreased in mTerc$^{-/-}$MMT mice compared to MMT and G0 mTerc$^{+/-}$MMT mice (Fig 1A). Fewer and smaller solid masses of tumors were observed in mTerc$^{-/-}$MMT mice (Fig. 1A). Similar results were observed in histological sections stained with H&E. Invasive tumors composed mainly of solid sheets of tumor cells were observed in MMT, mTerc$^{+/-}$MMT and, to a lesser extent, G1 mTerc$^{-/-}$MMT mice (Fig. 1B). By contrast, a more benign tumor pathology was observed in G2-G4 mTerc$^{-/-}$MMT mammary glands, even when these samples were harvested in older mice (Fig. 1B). For example, mammary tumor harvested in a G2 mTerc$^{-/-}$MMT mouse at the age of 20 weeks resembled to the late stage of premalignant lesions found in MMT mice at age of 8-9 weeks. The impaired tumorigenesis was more apparent in G3 and G4 mTerc$^{-/-}$MMT mice (Fig. 1B). Thus, tumor development in mTerc$^{-/-}$MMT mice is significantly impaired. The findings that the severity of such impairment is directly proportional to the number of generations of mTerc$^{-/-}$MMT mice suggests a critical role for telomere maintenance in mammary tumorigenesis.

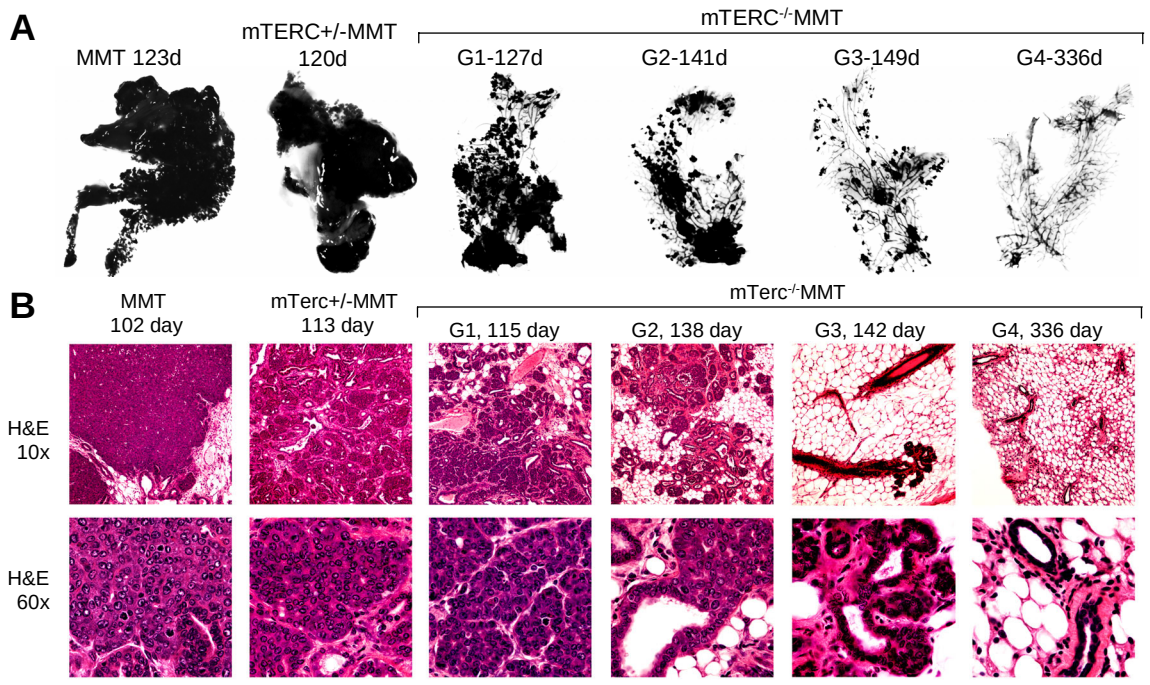

Figure 1. Tumor development delayed in mTerc$^{-/-}$MMT mice. (A) Tumor or mammary glands harvested from MMT, G0 mTerc$^{+/-}$MMT and G1 to G4 mTerc$^{-/-}$MMT mice at indicated age were processed for whole mount and stained with Carmine Alum. Whole mammary glands were photographed. The dark nodules are tumor masses. (B) The mammary glands and/or tumors from MMT, G0 mTerc$^{+/-}$MMT and G1 to G4 mTerc$^{-/-}$MMT mice harvested at indicated age were sectioned, stained with H&E and examined under microscope.

Inhibition of mammary tumors associated with telomere dysfunction. The results shown above indicate that an inability to maintain the telomere length may exert inhibitory effects in the tumorigenesis of mTerc$^{-/-}$MMT mice. To assess the relationship between tumor growth and telomere length, mammary tumors were harvested from MMT, G0 mTerc$^{+/-}$MMT and mTerc$^{-/-}$MMT (G1-G4) mice and assayed for telomerase activity and telomere length. As expected, telomerase activity was detected in the tumor samples from MMT and G0 mTerc$^{+/-}$MMT mice. In contrast, there was no detectable telomerase activity in the mammary tumor samples from mTerc$^{-/-}$MMT mice (Fig. 2A). In addition, progressive shortening of telomeres was observed in tumor cells from MMT, G0 mTerc$^{+/-}$MMT and mTerc$^{-/-}$MMT (G1-G4) mice (Fig. 2B). Thus deficiency of telomerase activity in mTerc$^{-/-}$MMT mice leads to progressive attrition of telomeres in mammary tumor cells. It is likely that critically shortened telomeres loss their protective function and may hamper mammary tumor formation. To assess this possibility, the frequency of anaphase bridge formation, a hallmark of chromosome end fusion caused by telomere dysfunction, was determined in the tumor cells from MMT, G0 mTerc$^{+/-}$MMT and mTerc$^{-/-}$MMT (G1-G4) mice. Indeed the frequency of anaphase bridges was significantly increased in G2 to G4 mTerc$^{-/-}$MMT mice (Fig. 2C and D),

suggesting that shortened telomeres in tumor cells from later generations of mTerc$^{-/-}$ MMT mice lose their protective function.

Collectively, these results indicate that the impairment of tumorigenesis in later generations of mTerc$^{-/-}$MMT mice is likely due to the inhibitory effects of telomerase inactivation and telomere dysfunction.

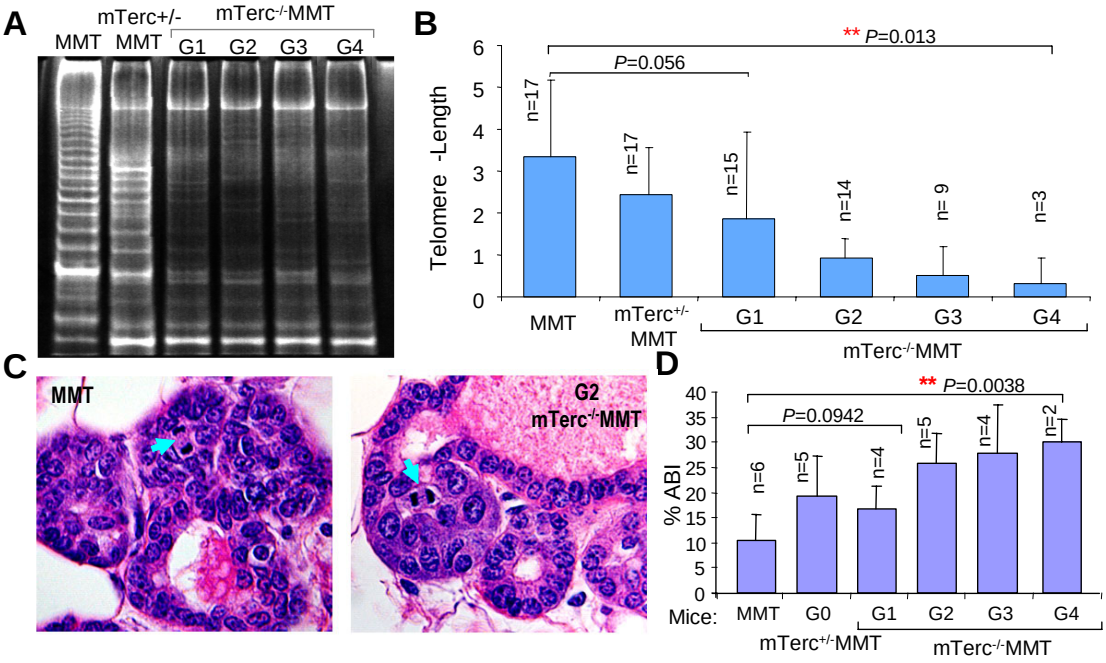

Figure 2. Telomerase activity and telomere dysfunction in tumor cells from mTerc$^{-/-}$MMT mice. (A) Determination of telomerase activity by TRAP in mammary tissues or tumors. Mammary glands and/or tumors from MMT, G0 mTerc$^{+/-}$MMT, G1 to G4 mTerc$^{-/-}$MMT mice were harvested. Telomerase activity was detected by TRAP. The PCR products (0.5 μg) were analyzed by 12.5 polyacrylamide nondenaturing gel and telomerase activity was measured using DNA silver staining kit. (B) Telomere length was determined by real-time PCR. Comparison of the average telomere length ratio (means ± SD) in MMT, G0 mTerc$^{+/-}$MMT and G1 to G4 mTerc$^{-/-}$MMT mice. The means of telomere length from G0 mTerc$^{+/-}$MMT and G1 to G4 mTerc$^{-/-}$MMT mice was compared with that of MMT group using Student *t*-test. (C-D) Anaphase bridge and anaphase bridge index (ABI) was analyzed in H&E stained sections of tumors and calculated as percentage of anaphases presenting chromatin bridges. A minimum of 30 anaphases were examined per tumor sample. The mTerc status/generation is indicated under each bar. The numbers of mice in each groups is also indicated. Arrows point cells in anaphase without bridge (C, left panel) or with chromatin bridge (C, right panel).

Effect of telomerase deficiency on tumor proliferation and inactivation of tumor cells. Telomerase deficiency and telomere dysfunction can result in impaired proliferation and increased apoptosis in tumor cells, leading to the impaired tumorigenesis. To determine the mechanism through which telomerase deficiency and telomere dysfunction exert inhibitory effect of mammary tumorigenesis, we examined

the proliferation and apoptosis in the mammary tumors from MMT, G0 mTerc$^{+/-}$MMT and G1 to G3 mTerc$^{-/-}$MMT mice using immunohistochemical staining with antibody against Ki67, a proliferation marker (11), and TUNEL assay (12). Whereas significant numbers of tumor cells from MMT and G0 mTerc$^{+/-}$MMT mice are positive for Ki67, suggesting robust proliferation for these cells, progressive decrease in Ki67-positive cells was observed in tumor cells from G1 to G3 mTerc$^{-/-}$MMT mice (Fig. 3A). In contrast to the impaired proliferation of tumor cells in mTerc$^{-/-}$MMT mice, increased apoptosis was observed in tumor cells from G1 to G3 mTerc$^{-/-}$MMT mice (Fig. 3B). Taken together, these results indicate that impaired proliferation and increased apoptosis in tumor cells from mTerc$^{-/-}$MMT mice are among the mechanisms through which telomerase deficiency and telomere dysfuntion exert inhibitory effect in mammary tumorigenesis.

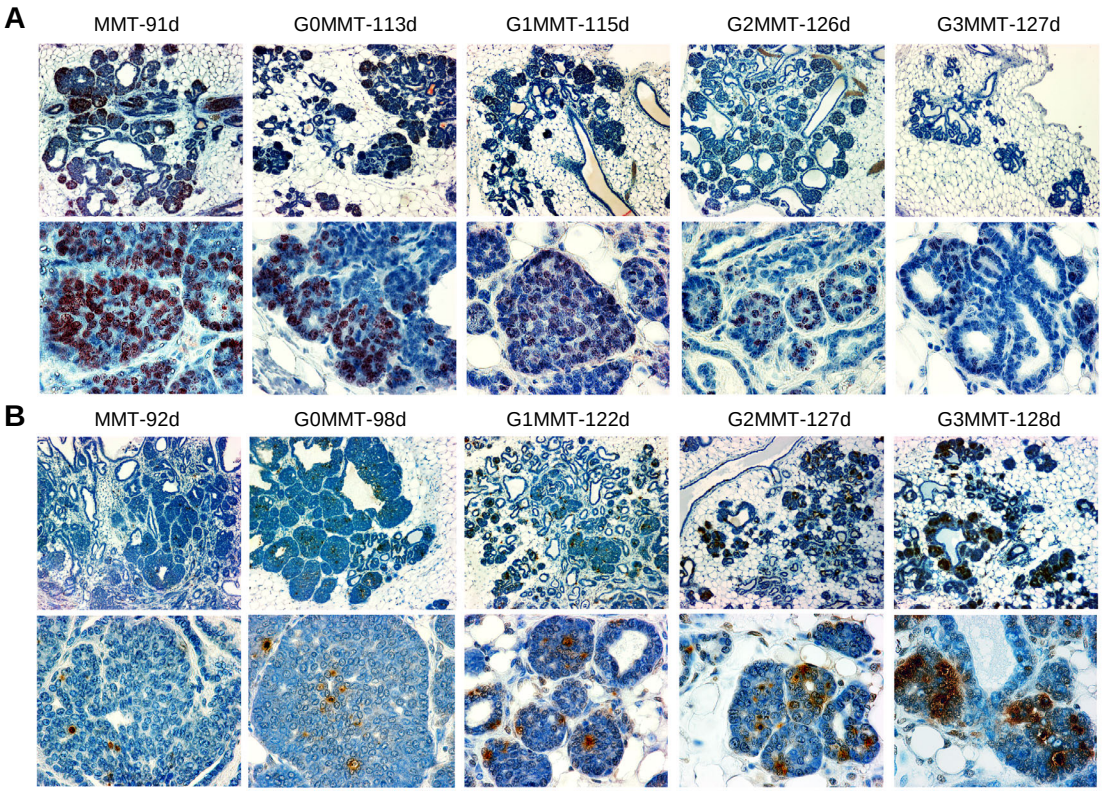

Figure 3. Proliferation and apoptosis of mammary carcinomas in telomerase-deficient MMT mice. (A) The mammary glands/tumors harvested from MMT, mTerc$^{+/-}$MMT and G1 to G3 mTerc$^{-/-}$MMT mice at indicated age were processed for frozen sections which were stained with anti-Ki67 at 1:20 dilution using immunohistochemical staining method. The red color indicates positive cells for Ki67 (10× or 60×). (B) TUNEL assay to detect the apoptotic cells. Cells stained with brown color are apoptotic cells (10× and 60×).

Effect of telomerase deficiency on reproductive systems. We next asked what is the systemic effect of telomerase deficiency on organs with high cellular proliferation. To address this question, we examined the spermatogenesis in the telomerase-null mice.

Histological examination of such testes revealed impairment of spermatogenesis in mTerc$^{-/-}$MMT mice, especially in the late generations of the mice. Testes from MMT and G0 mTerc$^{+/-}$MMT mice showed normal structure with well-developed seminiferous tubules, germ cells and supporting cells, whereas impairment of spermatogenesis was observed in G3 mTerc$^{-/-}$MMT mice (Fig. 4). In the testes of G3 mTerc$^{-/-}$MMT mice, the germ cells were markedly depleted and the lumen of tubules was lined by vacuolated Sertoli cells, suggesting a detrimental effect of telomerase deficiency and telomere dysfuntion on spermatogenesis in late generation of mTerc$^{-/-}$MMT mice.

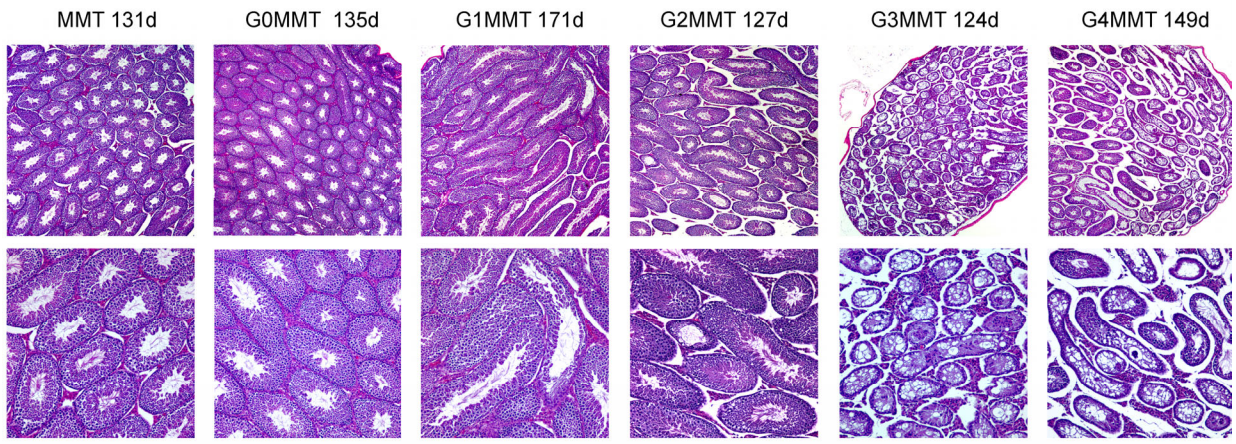

Figure 4. The effect of telomerase deficiency on the reproductive organs. The testes form MMT, G0 mTerc$^{+/-}$MMT and G1 and G3 mTerc$^{-/-}$MMT mice were prepared for sections that were stained with H&E. The slides were examined under microscope at 4x and 10x, respectively.

Task 2. **To determine the synergistic effect in the management of mammary carcinomas by combined immunotherapy and depletion of telomerase activity**

Effect of telomerase deficiency on the induction of antitumor immune response. Previous studies indicate that deficiency of telomerase activity renders tumor development in MMT mice delayed and tumor burden decreased, thus creating a favorable condition for immunoprevention and immunotreatment. To examine whether immunization in the background of telomerase deficiency can enhance the antitumor immunity, female mice from MMT, G0 mTerc$^{+/-}$MMT, G1 to G4 mTerc$^{-/-}$MMT groups were selected and immunized subcutaneously with fusions of DC and MUC1-positive mammary tumor cells (FC/MMT) as previously described (10). The vaccination was repeated every three to four weeks for up to five times. In the prevention study, the immunization of mice was initiated at age 2-4 weeks. In the treatment study, the immunization of mice started at age of 5-6 weeks.

Early vaccination of mice with FC/MMT delayed the appearance of mammary tumors. In the prevention study, female MMT, G0 mTerc$^{+/-}$MMT, G1 to G4 mTerc$^{-/-}$ MMT mice were randomly divided into two vaccinated and non-vaccinated groups. The mice were then examined for the appearance of mammary tumors. Delayed

appearance of mammary carcinomas was observed in mice vaccinated with FC/MMT compared with those non-vaccinated mice (Table 1). The time required for the mammary tumors in MMT, G0 mTerc$^{+/-}$MMT, G1, G2, G3 and G4 mTerc$^{-/-}$MMT mice that were vaccinated with FC/MMT were 124.7±3.8, 118.6±10.8, 139.9±25.3, 138±12.1, 146.8±21.2 and 215 days, respectively. The difference of time required for tumor development between mice with and without vaccination is statistically significant except for the G4 group due to the small sample size.

Immunization of mice at old age inhibited the development of mammary tumor. In the treatment study, the mice were immunized at age of 5-6 weeks. Table 1 shows that immunization with FC/MMT inhibited the progression of mammary tumors in the immunized mice. The time required for the mammary tumors in MMT, G0 mTerc$^{+/-}$MMT, G1 to G4 mTerc$^{-/-}$MMT mice that were immunized with FC/MMT were 112.4±10.4, 113.6±8.4, 136.3±10, 144.3±12.7, 158.7±15.7 and 247.3 days, respectively. The time required for tumor appearance was statistically different between the immunized and non-immunized mice in all groups except for G4 group.

Table-1 Tumor development in different groups of mTerc$^{-/-}$MMT mice with or without vaccination

Groups		MMT	mTerc$^{+/-}$ MMT (G0)	mTerc$^{-/-}$MMT mice			
				G1	G2	G3	G4
A	Mice (n)	14	15	5	14	9	2
	Tumor appearance (Days ± SD)	73.07 ± 28.03	96.07 ± 28.79	115.6 ± 8.88	117.14 ± 32.1	127.11 ± 35.84	225
B	Mice (n)	6	16	7	12	6	1
	Tumor appearance (Days ± SD)	124.67 ± 3.79	118.63 ± 10.84	139.86 ± 25.29	138 ± 12.05	146.83 ± 21.21	215
	P value	0.0094	0.010744	0.04754	0.003914	0.018558	n/a
C	Mice (n)	5	5	3	3	3	2
	Tumor appearance (Days ± SD)	112.4 ± 10.41	113.6 ± 8.38	136.33 ± 10.02	144.33 ± 12.66	158.67 ± 15.7	247.33
	P value	0.000353	0.04955	0.043117	0.009599	0.008011	n/a

A. Mice injected with PBS
B. Mice immunized with FC/MMT that was initiated at age of 2-4 weeks
C. Mice immunized with FC/MMT that was initiated at age of 5-6 weeks

Comparison of tumor development in littermates. To minimize the individual variation, we compared the latency time required for tumor appearance in littermates with or without immunization. In this experiment, at least one littermate was immunized and another one remains untreated. Their tumor development was monitored and compared. In the untreated groups, the time required for tumor appearance in MMT, G0 mTerc$^{+/-}$MMT and G1 to G3 mTerc$^{-/-}$MMT mice were 78.8±15.5, 108.8±8.9, 105.3±10.7, 114.3±14.3 and 125.6±10.9, respectively. By contrast, the latency time in

the immunized MMT, G0 mTerc$^{+/-}$MMT and G1 to G3 mTerc$^{-/-}$MMT mice were 112.4±10.4, 134±10.6, 128.8±10.3, 143.7±16.1 and 151.9±16 (Fig. 5A and B), respectively. Taken together, these results indicate that immunotherapy can further delay or inhibit the development of mammary tumors and comparable antitumor immunity is induced in the early generations of mTerc$^{-/-}$MMT mice. It should be noted that 24 mice (G1 mice, n=2 pair; G2 mice, n=2 pairs; G3 mice, n=3 pairs; G4 mice N=5 pairs) are still under observation due to the lengthy follow-up required for this project. For example, G3 or G4 mTerc$^{-/-}$MMT mice sometimes require more than 200 days observation for tumor development.

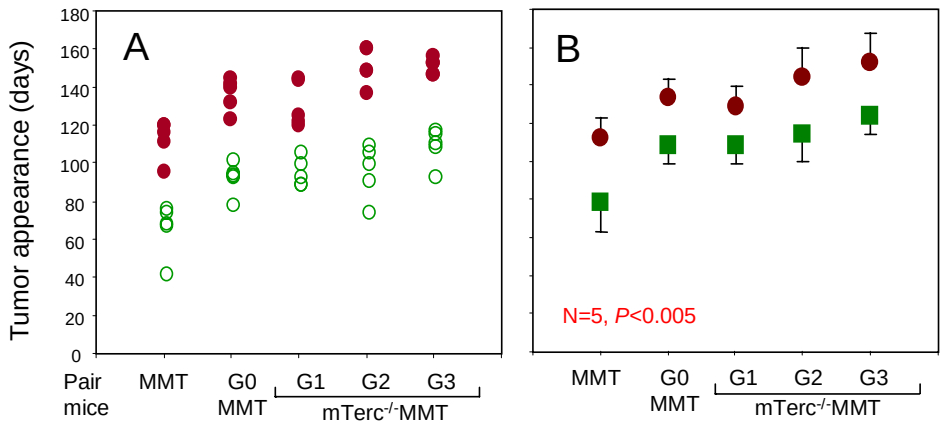

Figure 5. Comparison of latency time required for tumor development between littermates with or without immunization. (**A**) Time required for tumor appearance in MMT, G0 mTerc$^{+/-}$MMT and G1 to G3 mTerc$^{-/-}$MMT mice immunized with FC/MMT. Each symbol represents one mouse (n=5). (**B**) Average days needed for tumor appearance in littermates without immunization (■) or with immunization (●). The statistical analysis was determined by Student *t* test.

Effect of telomerase deficiency on proliferation of T cells. Previous studies indicate that telomerase activity and/or telomeres are involved in T cell proliferation (13). To assess whether T cell proliferation or division is affected in successive generations of mTerc$^{-/-}$MMT mice, we first measured T cell proliferation in mice with or without FC/MMT immunization using standard isotope incorporation. Minimal T cell proliferation was observed in lymph node cells (LNC) (Fig. 6A and C) or splenocytes (Fig. 6B and D) from non-immunized mice. In contrast, lymph node cells and splenocytes harvested from immunized MMT, G0 mTerc$^{+/-}$MMT, G1 and G2 mTerc$^{-/-}$MMT mice in the prevention (Fig. 6A and B) and treatment studies proliferated vigorously (Fig. 6C and D). We next assessed the division of T cells from MMT, G0 mTerc$^{+/-}$MMT and G1 to G3 mTerc$^{-/-}$MMT mice. We labeled the T cells with the fluorescent dye carboxyfluoroscein diacetate succinimidyl diester (CFSE) and then measured their division at the indicated times. CFSE is partitioned equally during cell division (14, 15), thus, this technique can measure the ability of T cell division. Both CD4 and CD8 T cells from immunized MMT mice divided vigorously (Fig. 5E and F). Similar results were obtained in CD4 and CD8 T cells from immunized G0 mTerc$^{+/-}$MMT, G1 or G2 mTerc$^{-/-}$MMT mice. Multiple rounds of cell division were observed in both CD4 and CD8 T cells from G0 mTerc$^{+/-}$MMT, G1

or G2 mTerc$^{-/-}$MMT mice immunized with FC/MMT. In contrast, fewer rounds of T cell divisions were observed from G3 mTerc$^{-/-}$MMT mice when compared with those from G1 and G2 mTerc$^{-/-}$MMT mice (Fig. 5E and F). Taken together, these results indicate that the ability of T cell proliferation or division is not affected in the early generations of mTerc$^{-/-}$MMT mice. However, decline of T cell division is observed in G3 mTerc$^{-/-}$MMT mice.

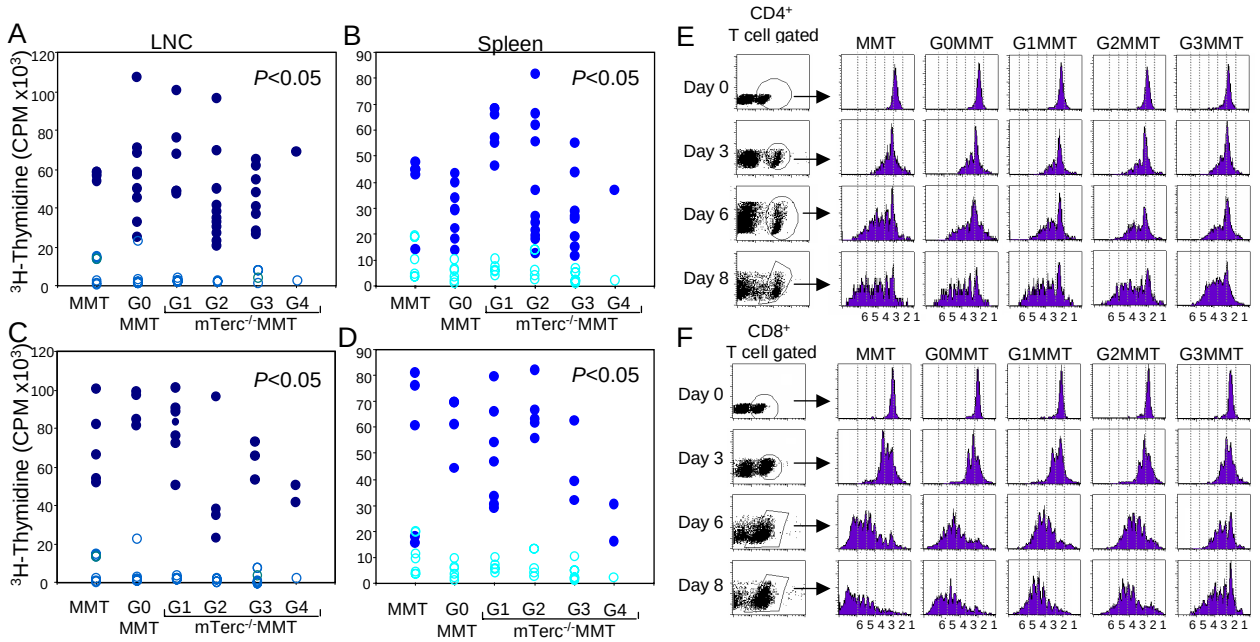

Figure 6. T cell proliferation assay. Cell proliferation of LNC (A and C) and splenocytes (B and D) obtained from mice immunized with FC/MMT (●) in the prevention study (A and B) or treatment study (C and D). The isolated LNC and splenocytes were cultured for 5 days. [^{3}H]thymidine was added during the last 16 hours of culture and the incorporation of [^{3}H]thymidine was measured. Each symbol represents the count from one mouse. The statistical analysis was determined by student *t*-test between immunized (●) and non-immunized mice (O). (E-F) Assessment of T cell division. LNC isolated from MMT and G0 mTerc$^{+/-}$MMT, G1 to G3 mTerc$^{-/-}$MMT mice immunized with FC/MMT were labeled with CFSE (Molecular Probes, Eugene, OR, final concentration 4 μM) for 10 min at 37^{o}C in a shaking water bath, and then washed twice and cultured in medium. On day 0, 3, 6, 8, the labeled LNC were collected, stained with PE-conjugated anti-CD4 or anti-CD8 mAb and analyzed by FACS. CD4 positive cells (E) and CD8 positive T cells (F) were gated and analyzed for cell division by FACS.

Effect of telomerase deficiency in the induction of CTL. Our previous studies show that immunization with FC/MMT induces CTL against mammary tumor cells in MMT mice (9, 10). To assess the effect of telomerase deficiency in the induction of CTL, mTerc$^{-/-}$MMT mice were immunized for 5 times at monthly intervals. The majority of mice were sacrificed at end of experiment. Their splenocytes or LNC were isolated and CTL activity was measured. CTL activity against mammary tumor cells was observed in both LNC (Fig. 7A and C) and splenocytes (Fig. 7B and D) from immunized but not

non-immunized mice. There is statistically significant difference in CTL activity between immunized and non-immunized mice (Fig. 7A-D). In addition, the CTL from immunized mice (Fig. 7F) were able to lyse the relevant tumor targets such as MC38/MUC1 or B16/MUC1 but not the irrelevant tumor target (B16), suggesting the specificity of CTL. In contrast, minimal CTL activity was detected in non-immunized mice (Fig. 7E). Collectively, these results show that comparable CTL can be induced in the first and second generations of mTerc$^{-/-}$MMT mice. However, decline of CTL in the late generation of mTerc$^{-/-}$MMT mice was observed.

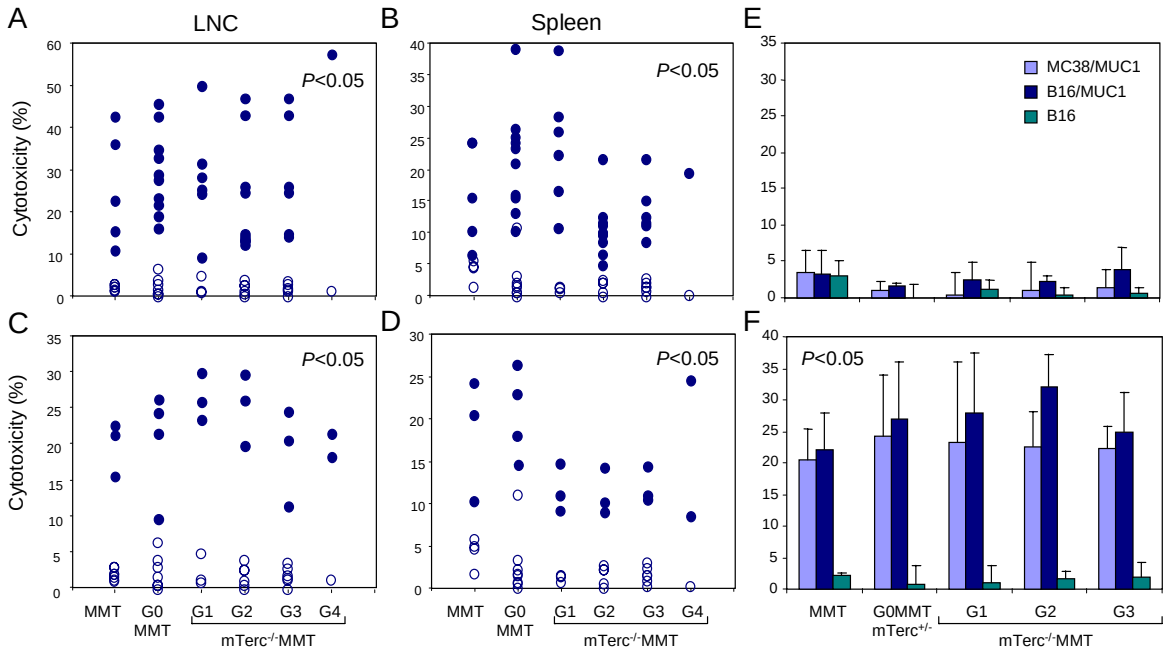

Figure 7. Induction of CTL. CTL response to FC/MMT vaccination in MMT, G0 mTerc$^{+/-}$MMT and G1 to G4 mTerc$^{-/-}$MMT mice. (A-D) Percentage of cytotoxicity of lymph node cells (A and C) or splenocytes (B and D) from non-immunized mice (O) or immunized mice (●). (A-B) Mice were immunized with 5×10^5 FC/MMT vaccine that was initiated at age of 2-4 weeks. (C-D) Mice immunized with 5×10^5 FC/MMT vaccine that was initiated at age of 5-6 weeks. The vaccination was repeated at the interval of 3-4 weeks. At the end of experiment, the mice were sacrificed and LNC and splenocytes were isolated and incubated with ^{51}Cr-labeled MMT tumor at 50:1 (LNC) E:T ratios or 100:1 (splenocytes) E:T ratios. CTL activity was determined by ^{51}Cr-release assay. (E-F) The mean CTL activity in mice without immunization (E) or with immunization (F) was presented. The MUC1-specificity of CTL activity were assessed by multiple targets including MC38/MUC1 and B16/MUC1 (both MUC1-positive), and B16 (MUC1-negative) tumor. Statistical significance was determined by X^2 test.

KEY RESEARCH ACCOMPLISHMENTS:

1. Successful generation of G4 mTerc$^{-/-}$MMT mice and increased production of MMT and G0 mTerc$^{+/-}$MMT, G1 to G4 mTerc$^{-/-}$MMT mice.

2. Telomerase inactivation has an inhibitory effect on the formation of mammary carcinomas. The inhibition of mammary tumors in G1 to G4 mTerc$^{-/-}$MMT mice is inversely correlated with the length of telomeres in the tumor cells, suggestion a critical role for telomere maintenance in tumorigenesis.

3. Total inactivation of telomerase has a detrimental effect on the function of mitotically active organs such as testes as demonstrated in G3 and G4 mTerc$^{-/-}$MMT mice.

4 Comparable induction of CTL activity in MMT, G0 mTerc$^{+/-}$MMT, G1 and G2 mTerc$^{-/-}$MMT mice by FC/MMT translated to prolonged latent time of mammary tumors. However, the CTL activity in late generations of mTerc$^{-/-}$MMT mice declined, suggesting that lack of telomerase activity may have a detrimental effect of CTL in the late generations of mTerc$^{-/-}$MMT mice.

5. The ability of T cell division in MMT, G0 mTerc$^{+/-}$MMT and G1 to G2 mTerc$^{-/-}$MMT mice is comparable as demonstrated by CFSE labeling and standard isotope incorporation. However, T cell division from G3 mTerc$^{-/-}$MMT mice shows sign of decline.

REPORTABLE OUTCOMES:

ABSTRACT

1. William Song, Chunlei Liu and Jianlin Gong. Role of telomerase and telemere maintenance in the development of mammary carcinomas. Conference Proceedings by AACR, A27, November 29, 2006

2. Baizheng Song, Chunlei Liu, Lindy Su and Jianlin Gong. Immunotherapy of spontaneous mammary carcinomas in a murine model deficient for telomerase activity. DOD Era of Hope 2008 meeting.

CONCLUSIONS:

1. We have successfully generated G0 mTerc$^{+/-}$MMT, G1, G2, G3 and G4 mTerc$^{-/-}$ MMT mice.

2. Inactivation of telomerase leads to progressive shortening of telomeres. Critically shortened telomeres lose the protective function of telomeres, resulting in the formation of anaphase bridge.

3. Progressive shortening of telomeres constraints the development of mammary tumors driven by PyMT oncogene.

4. Antitumor immunity can be induced in telomerase-null mice, resulting in further delay in the onset of mammary tumors in mice immunized with FC/MMT.

5. Comparable immune response to FC/MMT vaccination was observed in MMT, G0 mTerc$^{+/-}$MMT, G1 and G2 mTerc$^{-/-}$MMT mice including T cell proliferation and induction of CTL. However, the capacity for T cell division and induction of CTL from late generations of mTerc$^{-/-}$MMT mice was affected by the lack of telomerase activity and/or shortened telomeres.

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